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Karyotype Analysis of Eight Species and One Variety of *Carpesium* (Compositae) in Japan

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日本産ヤブタバコ属8種1変種の核型分析

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Chromosome number and karyotype of eight species and one variety of Japanese *Carpesium* were studied. Chromosome number 2n = 40 of *C. koidzumii* is reported for the first time. Karyotypes of the species studied fall into two groups on the basis of the number of the largest chromosomes.

The genus *Carpesium,* consisting of about 20 species of a perennial or biennial herb, is distributed in eastern Asia and Europe, especially in China, Korea and Japan. Eight species and one variety have been known from Japan (Kitamura et al. 1981). Concerning the Japanese species, karyological investigations are still insufficient, though Shimakawa (1952) and Arano (1962) have studied. Arano (1962) reported 2n = 40 chromosomes from six species and one variety, and 2n = 36 from *C. abrotanoides,* and then, classified them into five types based on karyological analysis. But Arano gave no description of karyological differences of these five types, particularly, the centromeric position and length of chromosomes. Therefore, I re-examined the chromosome number and karyotype of eight species and one variety of Japanese *Carpesium* including *C. koidzumii.*

Materials and methods

All of the plants examined in this study were collected from natural habitats. They were transplanted to the garden of Makino Herbarium (MAK), Tokyo Metropolitan University. The original source and number of individuals examined are shown in Table 1. Cytological preparation was made by the following procedure. Root tips were pretreated with 0.002M 8-hydroxyquinoline solution for about 3 hr at room temperature and fixed with 45% acetic acid for about 30 min. After being macerated in a 1:2 mixture of 45% acetic acid and 1N hydrochloric acid for 20 sec. at 60°C, root tips were stained with 2% acetic orcein solution for over one night. Subsequently, they were squashed with conventional method. The chromosome numbers and karyotypes were observed in well-spread metaphase plates of somatic root tip cells. The arm ratio (length of the long arm/length of the short arm)

Table 1. Chromosome number, locality and number of individuals examined.

Species	Chromosome no. (2n)	Locality	No. of ind. examined
C. abrotanoides	40	Tokyo Metropolitan Univ. campus	7
	40+2B	Namezawa, Tagata, Shizuoka Pref.	2
		Nippara, Nishitama, Tokyo	3
		Mt. Hinodesan, Nishitama, Tokyo	2
		Mt. Takaosan, Hachioji, Tokyo	1
C. rosulatum	40	Mt. Sanagisan, Numazu, Shizuoka Pref.	2
		Kannami, Tagata, Shizuoka Pref.	1
		Mt. Takaosan, Hachioji, Tokyo	3
C. glossophyllum	40	Mt. Sanagisan, Numazu, Shizuoka Pref.	1
		Tazima, Minamiaizu, Fukushima Pref.	2
		Chonan, Chosei, Chiba Pref.	2
		Mt. Mitakesan, Nishitama, Tokyo	2
		Mt. Takaosan, Hachioji, Tokyo	4
C. macrocephalum	40	Tatsuno, Kamiina, Nagano Pref.	1
		Mt. Kumotorisan, Nishitama, Tokyo	3
		Mt. Hinodesan, Nishitama, Tokyo	1
C. cernuum	40	Mt. Misakasan, Fujiyoshida, Yamanashi Pref.	3
		Umizawa, Nishitama, Tokyo	4
C. triste	40	Nikko, Tochigi Pref.	3
		Chitose, Hokkaido	2
		Nippara, Nishitama, Tokyo	4
C. divaricatum	40	Mt. Sanagisan, Numazu, Shizuoka Pref.	3
		Mt. Mitakesan, Nishitama, Tokyo	2
		Mt. Hinodesan, Nishitama, Tokyo	2
		Mt. Takaosan, Hachioji, Tokyo	7
C. divaricatum var. *matsuei*	40	Minakami, Tone, Gumma Pref.	1
	40+2B	Mt. Mitakesan, Nishitama, Tokyo	2
	40+3B	Mt. Kumotorisan, Nishitama, Tokyo	1
		Mt. Hinodesan, Nishitama, Tokyo	4
		Mt. Takaosan, Hachioji, Tokyo	1
C. koidzumii	40	Kadoma valley, Chiisagata, Nagano Pref.	1
		Mt. Misakasan, Fujiyoshida, Yamanashi Pref.	3

and the classification for the centromeric position on a chromosome used here are as follows; 1.0–1.7: metacentric, 1.7–3.0: submetacentric, 3.0–7.0: subtelocentric chromosome. The voucher specimens are deposited in Kyungpook National University Herbarium.

Results

The chromosome numbers and karyotypes counted in this study are presented in Table 1 and Figs. 1 & 2. The description of the karyotype of each species is as follows:

C. abrotanoides L. The chromosome number was counted as $2n=40$ and $40+2B$ in somatic cells (Fig. 1A), uncongruent with previous reports (Shimakawa 1952, Arano 1962; $2n=36$). The chromosomes were 4.9–1.0 μm in length and relatively smaller than those of the other species examined and their shape was more or less symmetrical. In its karyotype (Fig. 2A), a pair of the largest metacentric chromosome (nos. 1 & 2) were easily distinguished from the others. The karyotype included 20 metacentric (nos. 1 & 2, 9–14, 17–24, 27–30), 8 submetacentric (nos. 3–6, 15 & 16, 25 & 26), 2 subtelocentric (nos. 7 & 8) and other 10 chromosomes. The ten chromosomes were observed as meta- or submetacentric (nos. 31–40), although the centromeric position was not always visible. Two B-chromosomes were observed in chromosome complements of the individuals from Nippara, Tokyo and the populations in the campus, Tokyo Metropolitan University, Setagaya, Tokyo (Fig. 1A).

C. rosulatum Miq. The chromosome number was counted as $2n=40$ (Fig. 1B). Chromosome length was 4.3–1.5 μm and more or less symmetrical. In its karyotype (Fig. 2B), a pair of the largest submetacentric chromosomes (nos. 1 & 2) were easily distinguished from the others. The karyotype was composed of 14 metacentric (nos. 9 & 10, 25–30, 33–36, 39 & 40), 22 submetacentric (nos. 1 & 2, 5–8, 11–24, 37 & 38) and 4 subtelocentric chromosomes (nos. 3 & 4, 31 & 32).

C. glossophyllum Maxim. The chromosome number was counted as $2n=40$ (Fig. 1C). The chromosomes of this species were 5.6–1.7 μm in length and comparatively larger than those of the other taxa examined. They were somewhat symmetrical. A pair of the largest submetacentric chromosomes (nos. 1 & 2) were easily distinguished from the others in its karyotype (Fig. 2C). The karyotype was composed of 16 metacentric (nos. 7 & 8, 15 & 16, 21–26, 33–38), 16 submetacentric (nos. 1 & 2, 9 & 10, 13 & 14, 19 & 20, 27–32, 39 & 40) and 8 subtelocentric chromosomes (nos. 3–6, 11 & 12, 17 & 18).

C. macrocephalum Fr. et Sav. The chromosome number was counted as $2n=40$ (Fig. 1D). The chromosomes were 5.3–1.6 μm in length and relatively larger than those of the others and also more or less symmetrical. In its karyotype (Fig. 2D), two pairs of the largest metacentric chromosomes (nos. 1–4) were easily distinguished from the others. The karyotype was consisted of 30 metacentric (nos. 1–18, 21–28, 35–38), 8 submetacentric (nos. 19 & 20, 29–32, 39 & 40) and 2 subtelocentric chromosomes (nos. 33 & 34).

C. cernuum L. The chromosome number was counted as $2n=40$ (Fig. 1F). The chromosomes were 4.0–1.1 μm long and more or less symmetrical. Two pairs of the largest metacentric chromosomes (nos. 1–4) were easily distinguished from the others in its karyotype (Fig. 2E). The karyotype was composed of 28 metacentric (nos. 1–16, 23–26, 31–38), 8 submetacentric (nos. 17 & 18, 27–30, 39 & 40) and 4 subtelocentric chromosomes (nos. 19–22).

C. triste Maxim. The chromosome number was counted as $2n=40$ (Fig. 1E). The chromosomes were 3.5–1.1 μm long and relatively

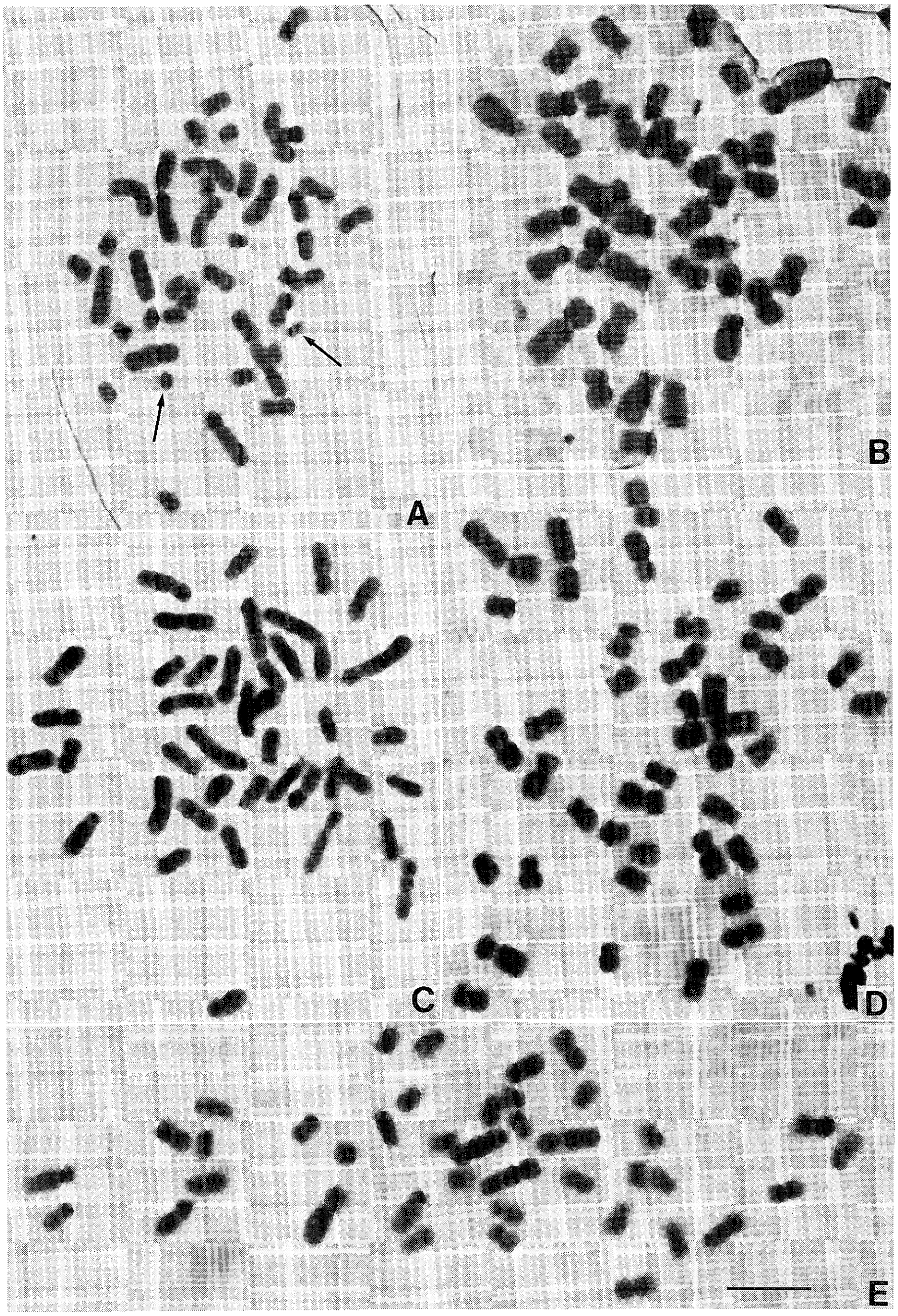

Fig. 1. Somatic metaphase chromosomes. A: *Carpesium abrotanoides* (2n=40+2B), B: *C. rosulatum* (2n=40), C: *C. glossophyllum* (2n=40), D: *C. macrocephalum* (2n=40), E: *C. triste* (2n=40), F: *C. cernuum* (2n=40), G: *C. divaricatum* (2n=40), H: *C. divaricatum* var. *matsuei* (2n=40+3B), I: *C. koidzumii* (2n=40). In A and H, arrows indicate the B-chromosomes, while in I, the small metacentric SAT-chromosomes. Bar indicates 5 μm.

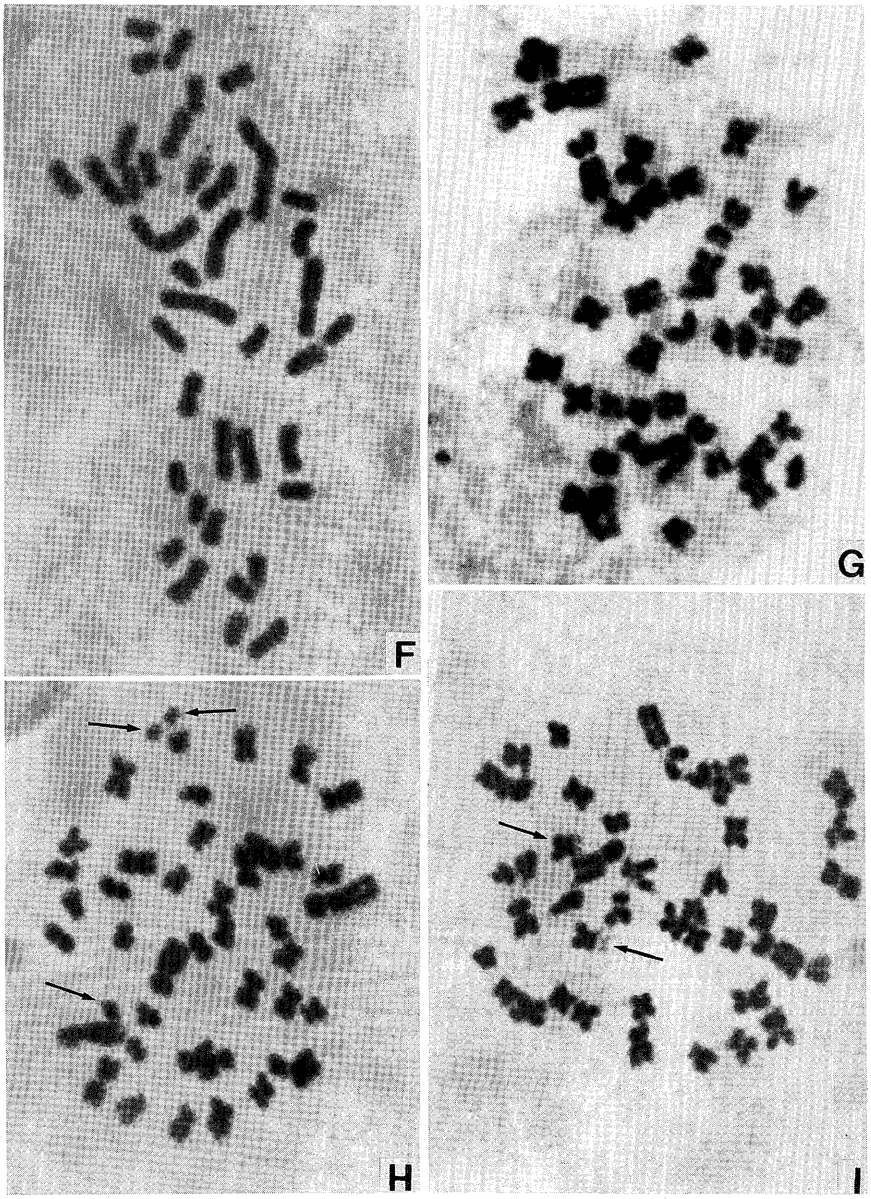

Fig. 1. (Continued)

symmetrical. In its karyotype (Fig. 2F), two pairs of the largest submetacentric chromosomes (nos. 1–4) were easily distinguished from the others. The karyotype was composed of 22 metacentric (nos. 7–10, 13 & 14, 17 & 18, 23 & 24, 29–40), 16 submetacentric (nos. 1–4, 11 & 12, 15 & 16, 19–22, 25–28) and 2 subtelocentric chromosomes (nos. 5 & 6).

C. divaricatum Sieb. et Zucc. The chromosome number was counted as $2n=40$ (Fig. 1G). The chromosomes were 3.6–1.1 μm long and more or less symmetrical. In its karyotype (Fig. 2G), two pairs of the largest submetacentric chromosomes (nos. 1–4) were easily distinguished from the others. The karyotype was composed of 26 metacentric (nos. 5–8, 13–16, 21–32, 35–40),

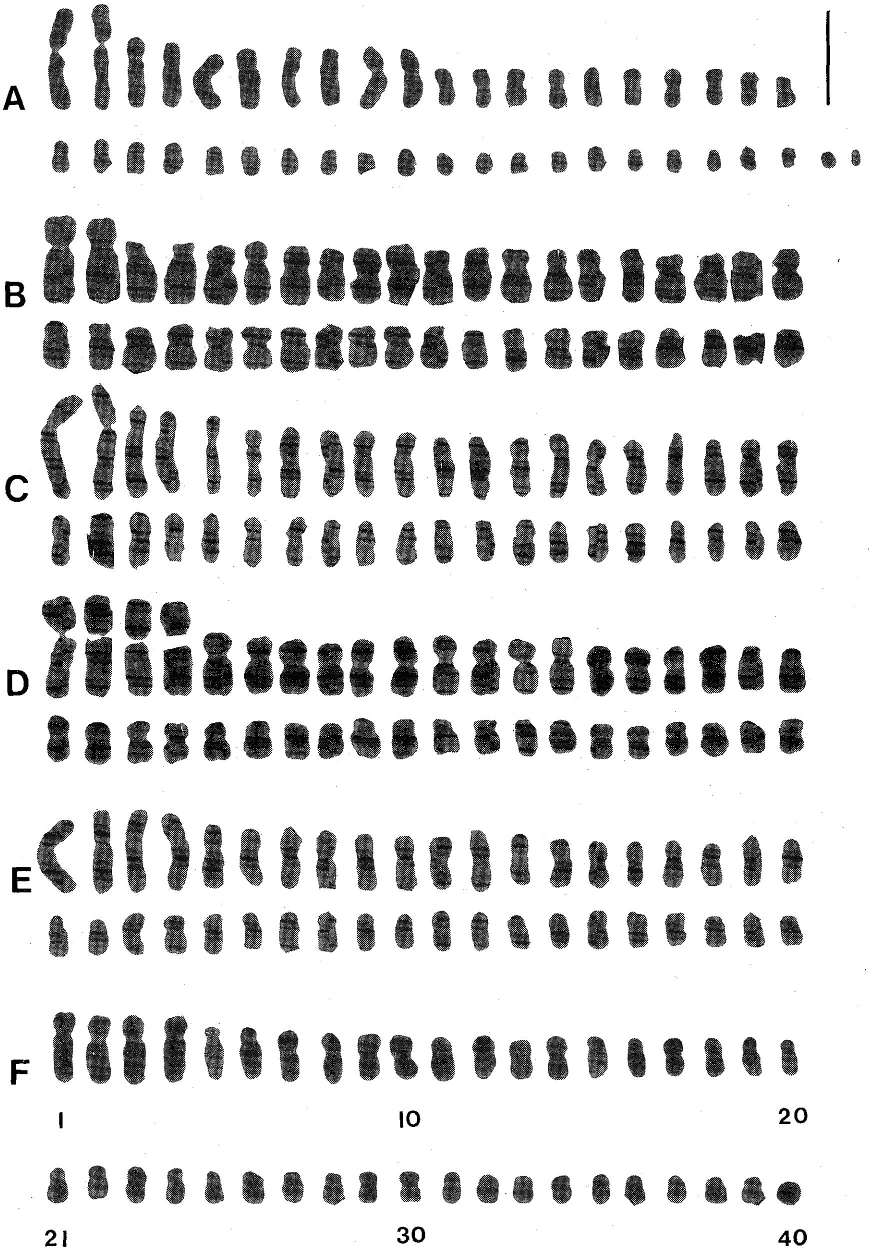

Fig. 2. Serial arrangement of the somatic metaphase chromosomes. A: *Carpesium abrotanoides* (2n=40+2B), B: *C. rosulatum* (2n=40), C: *C. glossophyllum* (2n=40), D: *C. macrocephalum* (2n=40), E: *C. cernuum* (2n=40), F: *C. triste* (2n=40), G: *C. divaricatum* (2n=40), H: *C. divaricatum* var. *matsuei* (2n=40+3B), I: *C. koidzumii* (2n=40). Arrows in I indicate the small metacentric SAT-chromosomes. Bar indicates 5 μm.

10 submetacentric (nos. 1–4, 9 & 10, 17–20) and 4 subtelocentric chromosomes (nos. 11 & 12, 33 & 34).

C. divaricatum var. *matsuei* Kitam. The chromosome number was counted as 2n=40, 40+2B and 40+3B (Fig. 1H) coincident with

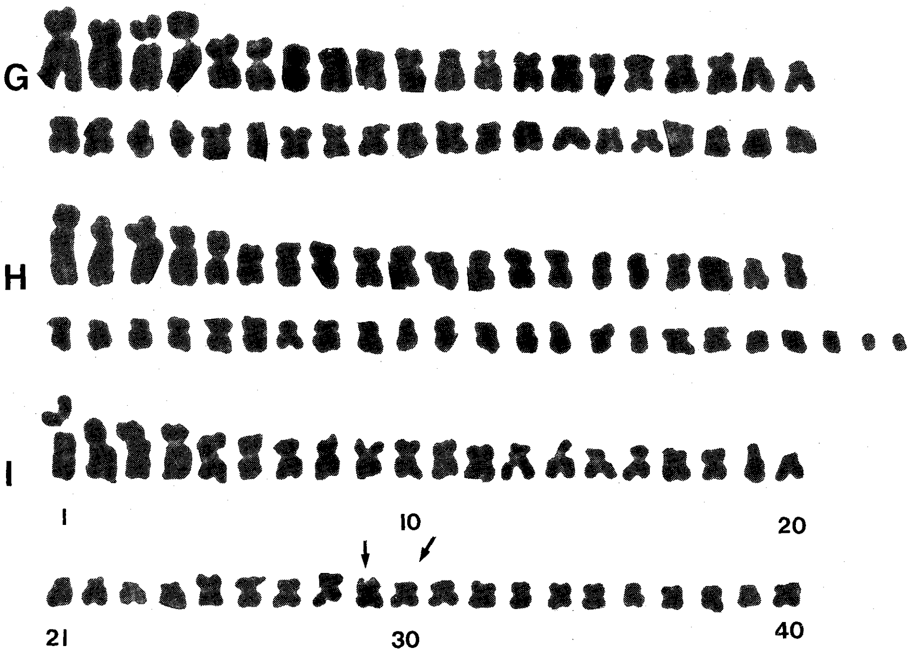

Fig. 2. (Continued)

previous report (Arano, 1962) except existence of B-chromosome. The length of the chromosomes was 3.6–1.1 μm and their shape was relatively symmetrical. Two pairs of the largest submetacentric chromosomes (nos. 1–4) were easily distinguished from the others in its karyotype (Fig. 2H). The karyotype was consisted of 24 metacentric (nos. 5 & 6, 9 & 10, 13–18, 25–32, 35–40), 12 submetacentric (nos. 1–4, 7 & 8, 11 & 12, 23 & 24, 33 & 34) and 4 subtelocentric chromosomes (nos. 19–22). Two or three B-chromosomes were observed in one individual from Mt. Takaosan population, Tokyo (Fig. 1H).

C. koidzumii Makino The chromosome number was counted as 2n = 40 (Fig. 1I) for the first time. The chromosomes of this species were 3.4–1.1 μm in length and comparatively smaller than those of the others, and more or less symmetrical. In its karyotype (Fig. 2I), two pairs of the largest submetacentric chromosomes (nos. 1–4) were recognized. The karyotype was composed of 26 metacentric (nos. 5–12, 17 & 18, 25–40), 10 submetacentric (nos. 1–4, 13–16, 23 & 24) and 4 subtelocentric chromosomes (nos. 19–22). A SAT-chromosome was observed on the short arm of two metacentric chromosomes (nos. 29 & 30), although it was not always confirmed.

Discussion

Among the eight species and one variety of *Carpesium*, no great difference was observed in their karyotype; all having 2n = 40 chromosomes that are more or less symmetrical, although slight differences are recognized among their chromosomal complements (Fig. 2). According to Shimakawa (1952) and Arano (1962), *C. abrotanoides* was reported as 2n = 36 in chromosome number, but there is a possibility of miscounting because the chromosomes are too small. In *Carpesium*, two or four largest chromosomes with median or submedian centromeres are recognized in general in a karyotype.

Therefore, the karyotypes can be classified into two groups. The first group including *C. abrotanoides, C. rosulatum* and *C. glossophyllum* has a pair of the largest chromosomes (nos. 1 & 2). This group can be divided into two subgroups by the centromeric position of the largest chromosome pair. One of these subgroups includes *C. abrotanoides*; the largest pair are metacentric and all of the chromosomes are relatively discontinuous in size. This species is a biennial herb and the only species having sessile heads among the species examined. The other subgroup (*C. rosulatum* and *C. glossophyllum*) can be distinguished from the former in having a submetacentric pair of the largest chromosomes and the chromosomes are more or less continuous in length. In addition, these two species have similar karyotype, especially in meta- or submetacentric chromosomes. In morphological features, rosettes consisting of radical leaves persist at the flowering time.

The second group including *C. macrocephalum, C. cernuum, C. triste, C. divaricatum, C. divaricatum* var. *matsuei* and *C. koidzumii* has two pairs of the largest chromosomes which are meta- or submetacentric (nos. 1–4). This group can be divided into three subgroups based on their karyotypes. The first subgroup includes *C. macrocephalum* and *C. cernuum*; the largest pairs are metacentric (nos. 1–4) and the others are mostly metacentric. These two species have winged petioles and semi-globose heads which are relatively large in size. The second subgroup including *C. triste* is characterized by four large submetacentric (nos. 1–4) and two large subtelocentric chromosomes (nos. 5 & 6). In addition, this species is distributed in relatively high altitude comparing with the other species and its radical leaves persist partially at the flowering time. The third subgroup, which includes *C.*

Table 2. Karyotypes and morphological characteristics of Japanese *Carpesium.*

Species	m	sm	st	m-sm	No. of largest chromosomes	Karyotypes	Morphological characteristics
C. abrotanoides	20	8	2	10	2(m)	A_1	Sessile heads
C. rosulatum	14	22	4	•	2(sm)	A_2	Rosette radical leaves
C. glossophyllum	16	16	8	•	2(sm)		
C. macrocephalum	30	8	2	•	4(m)	B_1	Winged petioles & half-globose heads
C. cernuum	28	8	4	•	4(m)		
C. triste	22	16	2	•	4(sm)	B_2	Semirosette radical leaves
C. divaricatum	26	10	4	•	4(sm)	B_3	Wingless petioles & ovate or ovate-oblong low leaves
C. divaricatum var. *matsuei*	24	12	4	•	4(sm)		
C. koidzumii	26	10	4	•	4(sm)		

divaricatum, C. divaricatum var. *matsuei* and *C. koidzumii,* is characterized by four large submetacentric chromosomes (nos. 1–4) and four small subtelocentric chromosomes in the karyotypes. These three species have commonly ovate or oblong-ovate lower leaves which are abruptly narrowed on wingless petioles.

Karyotypes and morphological characteristics of Japanese *Carpesium* are summarized in Table 2. Meiotic studies and more detail karyotype investigations will help to demonstrate the relationship among these species.

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Endnote

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要　旨

日本産ヤブタバコ属 *Carpesium* 植物8種1変種について核型分析の再検討を試みた．従来，未解析であったホソバガンクビソウ *C. koidzumii* において，2n＝40の染色体数が確認された．またヤブタバコ *C. abrotanoides* は，従来2n＝36と報告されてきたが，観察したすべての個体が2n＝40で，2n＝36のものは確認できなかった．核型分析より，この属の諸種は染色体組を構成する特に長い染色体の数によって，大きく2つの群に分けることができた．1つは2本の長い染色体をもつ群で，ヤブタバコ，ヒメガンクビソウ *C. rosulatum* 及びサジガンクビソウ *C. glossophyllum* である．もう1つは4本の長い染色体をもつ群で，オオガンクビソウ *C. macrocephalum*，コヤブタバコ *C. cernuum*，ガンクビソウ *C. divaricatum*，ノッポロガンクビソウ *C. divaricatum* var. *matsuei* 及びホソバガンクビソウが属する．この2つの群は長い染色体の形態によって，それぞれ2つ及び3つの亜群に分けることができる．